

# Rapid $^{14}\text{C}$ analysis of dissolved organic carbon in non-saline waters

*Susan Q. Lang<sup>1,2</sup>, Cameron P. McIntyre<sup>3,4</sup>, Stefano M. Bernasconi<sup>1</sup>, Gretchen Fröh-Green<sup>1</sup>,  
Britta M. Voss<sup>5,6</sup>, Timothy I. Eglinton<sup>1</sup>, Lukas Wacker<sup>3</sup>*

<sup>1</sup>Department of Earth Sciences, ETH Zürich, 8092 Zürich, Switzerland

<sup>2</sup>Current address: Department of Earth and Ocean Sciences, University of South Carolina,  
Columbia, SC 29208

<sup>3</sup>Laboratory of Ion Beam Physics, ETH Zürich, 8093 Zürich, Switzerland

<sup>4</sup>Current address: Scottish Universities Environmental Research Centre, Glasgow, Scotland

<sup>5</sup>Woods Hole Oceanographic Institution, Department of Marine Chemistry & Geochemistry,  
Woods Hole, MA, USA 02543

<sup>6</sup>Massachusetts Institute of Technology, Department of Earth, Atmospheric & Planetary Sciences,  
Cambridge, MA, USA 02139

## Abstract:

The  $^{14}\text{C}$  content of dissolved organic carbon (DOC) in rivers, lakes, and other non-saline waters can provide valuable information on carbon cycling dynamics in the environment. DOC is typically prepared for  $^{14}\text{C}$  analysis by accelerator mass spectrometry (AMS) either by UV oxidation or by freeze-drying and sealed tube combustion. We present here a new method for the rapid analysis of  $^{14}\text{C}$  of DOC using wet chemical oxidation (WCO) and automated headspace sampling of  $\text{CO}_2$ . The approach is an adaption of recently developed methods using aqueous persulfate oxidant to determine the  $\delta^{13}\text{C}$  of DOC in non-saline water samples and the  $^{14}\text{C}$  content of volatile organic acids. One advantage of the current method over UV oxidation is higher throughput: 22 samples and 10 processing standards can be prepared in one day and analyzed in a second day, allowing a full suite of  $^{14}\text{C}$  processing standards and blanks to be run in conjunction with samples. A second advantage is that there is less potential for cross-contamination between samples.

## Introduction

The  $^{14}\text{C}$  content of dissolved organic carbon (DOC) is a powerful tool for distinguishing sources and inputs of organic matter in aquatic systems. Currently, DOC is prepared for  $^{14}\text{C}$  analysis by accelerator mass spectrometry (AMS) using one of three offline methods. With the first method, samples are oxidized on a vacuum line using ultraviolet light (e.g. Armstrong et al., 1966; Williams 1968; Bauer et al., 1998; Druffel et al., 1989; Beaupré et al., 2007). UV oxidation has the advantages of extremely low blanks, the ability to analyze saline samples, and large enough volumes (~1 liter) to generate sufficient  $\text{CO}_2$  even for samples with low concentrations of carbon. It has the disadvantage that samples are analyzed at a rate of approximately 1-2 per day. In a somewhat similar approach, potassium permanganate instead of UV Oxidation has been used to convert organic matter to  $\text{CO}_2$  in large reactors (500 mL). Two DOC samples can be evaporated and reacted on one vacuum line, then the  $\text{CO}_2$  subsequently extracted, purified, and trapped on a second vacuum line (Leonard et al, 2013). With the third method, samples are freeze-dried in quartz tubes and combusted to  $\text{CO}_2$  in the presence of cupric oxide, in a similar fashion to solid organic carbon samples. The  $\text{CO}_2$  generated by this closed tube combustion (CTC) is then either graphitized for analysis on an AMS or is characterized directly with a gas source AMS (Palmer et al., 2001; Neff et al., 2006; Mann et al., 2015). Multiple samples can be prepared simultaneously (subject to number of available ports on the vacuum line), with the time from initial freeze-drying to loading on the AMS taking approximately 3 days.

We present here a new method for the analysis of  $^{14}\text{C}$  content of non-saline DOC samples that is based on two recently established protocols. The  $\delta^{13}\text{C}$  analysis of DOC using wet chemical oxidation (WCO) in 12 mL gas-tight Exetainer® vials was recently developed so that samples could be loaded into an automated headspace sampler interfaced with an isotope ratio mass spectrometer (Lang et al., 2012). The method has the benefit of low blanks and short preparation times, although it is not amenable to saline fluids as chloride interferes with the persulfate oxidation. This oxidation approach was subsequently applied to the compound-specific  $^{14}\text{C}$  analysis of the individual volatile organic acids formate and acetate (Lang et al., 2013). The compounds were isolated by high performance liquid chromatography, collected in Exetainer® vials, and chemically oxidized to  $\text{CO}_2$ . The vials were then loaded into an automated headspace sampler interfaced with an AMS (Fahrni et al., 2013). The current procedure combines these previous methods, and demonstrates that non-saline DOC samples, such as those from rivers or lakes, can be similarly analyzed. The method was verified using standards of known isotopic composition, and with freshwater environmental samples that had also been previously analyzed by either UV-oxidation at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) or by CTC at ETH-Zürich.

## Methods

### *Collection of Environmental Samples:*

Fraser River samples were collected in 2009 from Fort Langley British Columbia (49.172°N; -122.577°E). They were filtered through an in-line capsule filter (Pall AcroPak 500 Supor membrane, 0.2 µm size with 0.8 µm pre-filter; as in Voss et al., 2015) and acidified in the field to pH 2 with ACS certified 85% H<sub>3</sub>PO<sub>4</sub> into pre-combusted amber glass bottles with acid washed caps and stored in the dark at room temperature. These samples were prepared for <sup>14</sup>C analysis using both UV oxidation at NOSAMS in 2010 and wet chemical oxidation at ETH-Zürich in 2014.

Arctic water samples from the Kolyma River Basin were collected in September of 2012. Water samples were collected from the main stem of the Kolyma River (“arctic stream”) approximately 2 km upstream from Chersky, Russia, and from a small-order permafrost thaw stream (“permafrost stream”) which drained from an exposure known as Duvanni Yar (Spencer et al., 2015; Mann et al., 2015). Samples were filtered through pre-combusted (450°C) GF/F glass fiber filters to remove particles and stored frozen in acid-washed high-density polyethylene bottles (Spencer et al., 2015; Mann et al., 2015). These samples were prepared for <sup>14</sup>C analysis at ETH-Zürich using both freeze-drying/CTC (June, 2013) and wet chemical oxidation (2013 and 2014, Table 2).

### *UV Oxidation, NOSAMS:*

Dissolved organic carbon was oxidized using ultraviolet light by the method of Beaupré et al. (2007). A 50-60 g aliquot of sample was added to pre-oxidized Milli-Q water to bring concentrations into the normal working range of the system. The evolved CO<sub>2</sub> was stripped from water and cryogenically collected, then reduced into graphite with the use of a catalyst in the presence of excess hydrogen gas. The graphite was pressed into target cartridges and analyzed for <sup>14</sup>C by AMS at NOSAMS.

### *Freeze-Drying, ETH-Zürich:*

Frozen Arctic water samples were thawed and an aliquot was transferred to pre-combusted (850°C for 5 h) quartz tubes. Water was removed by freeze-drying and samples were fumigated with acid to remove carbonate. Pre-combusted CuO was added to the tubes, which were subsequently flame sealed under vacuum. Organic carbon was converted to CO<sub>2</sub> by heating the vials to 850°C for 6 hours. The evolved CO<sub>2</sub> was cryogenically quantified, sealed into a glass tube, and loaded for <sup>14</sup>C analysis into the MICADAS (Mini Carbon Dating System) at the Laboratory of Ion Beam Physics, ETH-Zürich (Wacker et al., 2010; Wacker et al., 2013; Molnár et al., 2013).

### *Wet Chemical Oxidation, ETH-Zürich*

The wet chemical oxidation approach was modified from one recently developed to determine δ<sup>13</sup>C values of DOC in non-saline water samples (Lang et al., 2012). The integration between organic compounds oxidized in Exetainer® vials and the AMS was adapted from a method to

determine the  $^{14}\text{C}$  content of volatile organic acids (Lang et al., 2013). In brief, samples were transferred into 12 mL Exetainer® screw-capped vials with butyl rubber septum (Labco, Buckinghamshire, United Kingdom, P/N 938W). A 1 mL aliquot of acidified sodium persulfate solution (100 mL  $\text{H}_2\text{O}$  + 4.0 g  $\text{Na}_2\text{S}_2\text{O}_8$  + 200  $\mu\text{L}$  of 85%  $\text{H}_3\text{PO}_4$ ) was added as an oxidant and samples were sealed and purged with high-purity helium gas (Grade 5.0, 99.9999% pure, for 8 minutes at  $>100$  mL/minute) to eliminate inorganic  $\text{CO}_2$  from the vial. The samples were then heated to  $95^\circ\text{C}$  for one hour to convert any sample DOC to  $\text{CO}_2$ . All glassware was pre-combusted at  $500^\circ\text{C}$  for 5 hours to remove organic contaminants. Further specifics on optimizing the oxidation conditions and minimizing processing blanks can be found in Lang et al. (2012).

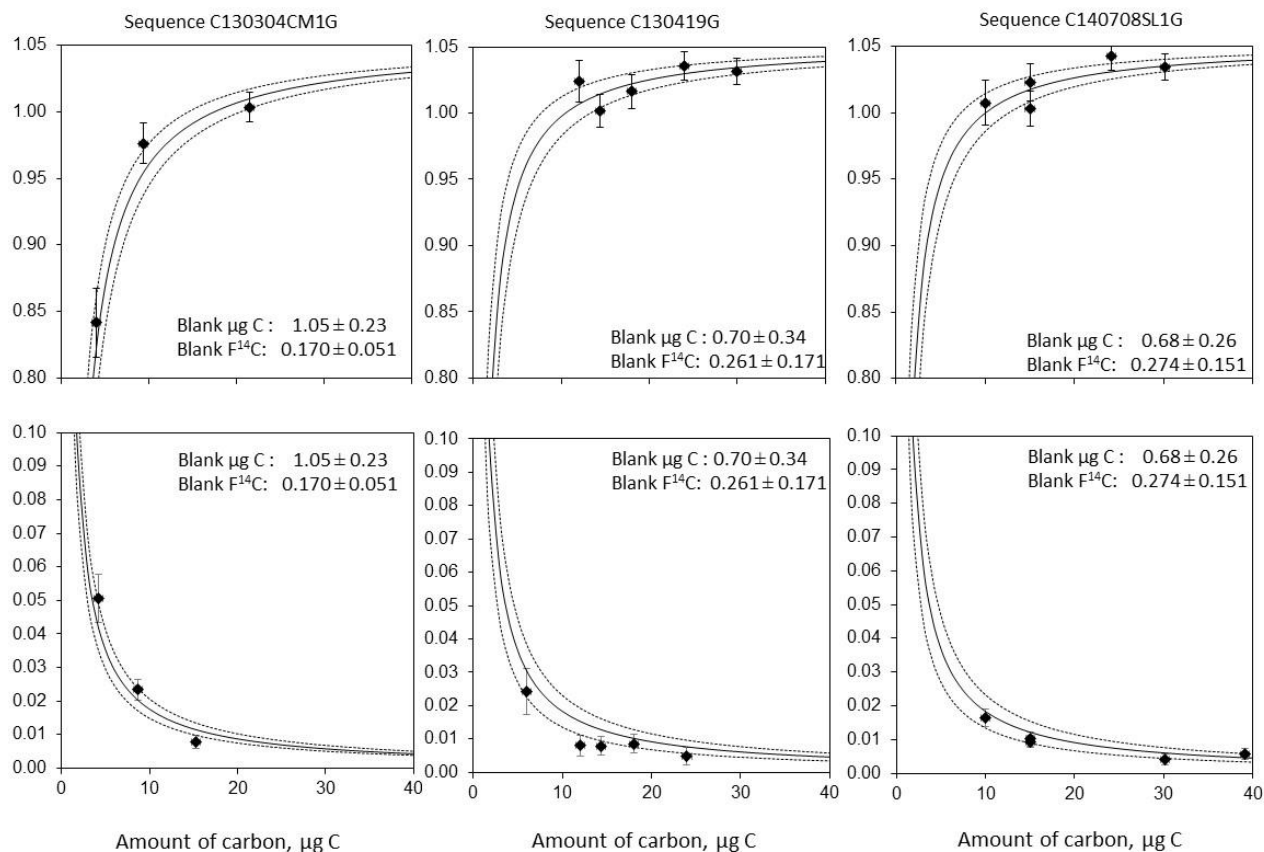
To determine the  $^{14}\text{C}$  content of the evolved  $\text{CO}_2$ , the samples were loaded into the carbonate handling system of the MICADAS accelerator mass spectrometer (AMS) equipped with a gas-accepting ion source (GIS) (Ruff et al., 2007; Wacker et al., 2013; Molnár et al., 2013). This gas transfer system automatically moves the  $\text{CO}_2$  in septum-sealed vials over a magnesium perchlorate water trap onto a X13 zeolite molecular sieve (sodium aluminosilicate) at room temperature. The zeolite trap is then rapidly heated to  $450^\circ\text{C}$  to release the  $\text{CO}_2$ , which is then transferred to a gas-tight syringe. An appropriate amount of helium is added to the syringe to dilute the gas to a 5 % v/v  $\text{CO}_2$  in helium, and the plunger is depressed slowly to feed the mixture into the GIS at a constant rate. The carbonate handling system was modified with the addition of a sparging needle to strip any  $\text{CO}_2$  dissolved in the water. A second, shorter needle carried the displaced sample  $\text{CO}_2$  gas from the vial headspace to the zeolite trap. Further specifics on the coupling of the Exetainer® samples to the AMS can be found in Molnár et al. (2013), Wacker et al. (2013), and Lang et al. (2013).

The raw  $^{14}\text{C}$  data are reported as fraction modern ( $F^{14}\text{C}$ ) after Reimer et al., (2004), and after correction for instrumental background, standard normalization, and evaluation of uncertainty using the software program BATS (Wacker et al., 2010). An additional correction was made for contamination introduced during the isolation and oxidation procedures (the processing blank), as detailed below.

A batch of 22 samples and 10 standards can be prepared in approximately 4-6 hours. Transferring the samples and standards into clean Exetainer® vials and adding the oxidant requires 1-2 hours. Two vials can be flushed with helium simultaneously, with the batch completely purged within  $\sim 2.5$  hours. All vials then react on the block heater for 1 hour. The helium flushing time is the rate determining step since samples can be transferred while this is ongoing; adding additional purging stations and/or automating this step would further reduce preparation times. Typically, samples were allowed to cool to room temperature overnight before loading them onto the AMS autosampler. Once the AMS had been focused and pure gas standards had been analyzed for calibration, the batch of 32 samples plus standards could be processed within  $\sim 4$  hours.

### Verification Approach

Two approaches were used to verify the method. First, two powdered standards with known  $F^{14}C$  signatures were dissolved in high purity Milli-Q water over a range of concentrations and analyzed for  $F^{14}C$  content. The two standards, phthalic acid (Sigma Aldrich P/N 8001-100g,  $\geq 99.5\%$  purity, Lot 1431342V,  $\delta^{13}C = -12.4\text{‰}$ ,  $F^{14}C < 0.0025$ , ETH-47292) and sucrose (Sigma Aldrich P/N S7903-250g,  $\geq 99.5\%$  purity, Lot 090M02112V,  $\delta^{13}C = -33.6\text{‰}$ ,  $F^{14}C = 1.053 \pm 0.003$ , ETH-47293) were chosen for their distinct isotope signatures, solubility in water and, in the case of phthalic acid, and chemical recalcitrance. The standards were prepared in 4 mL of Milli-Q water in concentration ranges from 83 – 833  $\mu\text{mol C/L}$ , corresponding to 4 – 40  $\mu\text{g}$  of organic carbon total (Figure 1). This range was chosen to represent the approximate concentrations of DOC in rivers and lakes and to cover the lower end of the MICADAS sample size capacity. The generated data were used to both verify the method and to determine the size and isotopic composition of the blank.



**Figure 1.**  $F^{14}C$  vs  $\mu\text{g C}$  of sucrose (upper panels,  $F^{14}C = 1.053 \pm 0.003$ ) and phthalic acid (lower panels,  $F^{14}C < 0.002$ ). The solid line in both panels represents the idealized mixture between the standards and a blank with characteristics determined for that particular run (see Table 1). Dashed lines represent 95% confidence intervals. Individual markers are not corrected for blanks; the y-axis error bars represent the instrument error only ( $\pm 1 \sigma$ ).

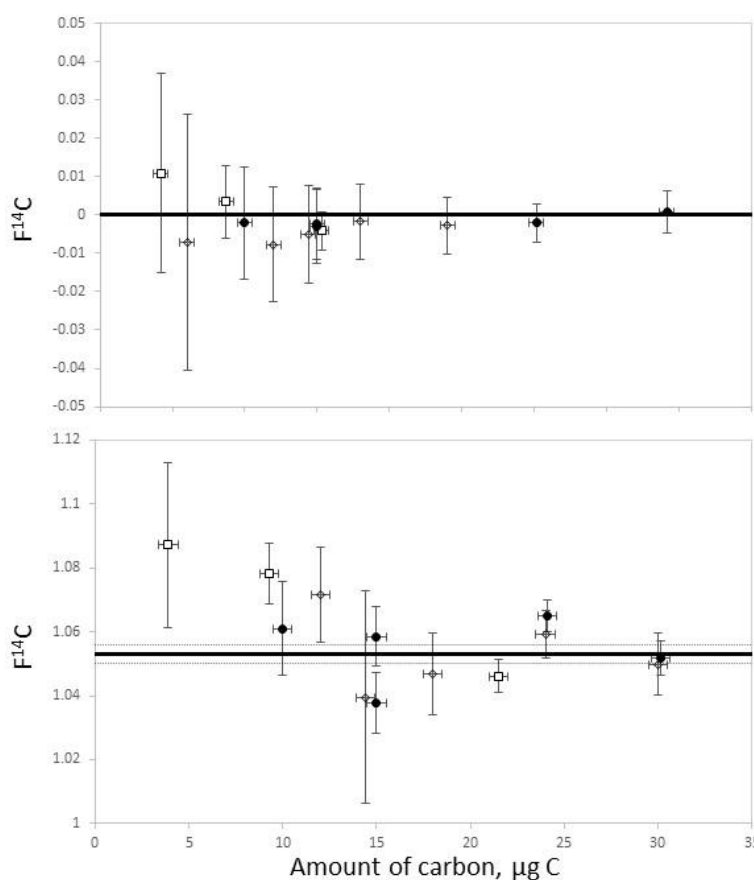
Second, riverine samples from the Fraser River and the Arctic were analyzed by persulfate oxidation and compared to the  $F^{14}C$  values determined on the same samples by other means, either UV oxidation (NOSAMS) or freeze-drying and closed tube combustion (ETH). The raw  $F^{14}C$  data generated from the riverine samples were corrected for the presence of a blank using the sucrose and phthalic acid standards.

## Results and Discussion

### *Standards with known $F^{14}C$ content*

Radiocarbon analysis of organic matter is highly sensitive to contributions from extraneous carbon and, since this extraneous carbon is frequently too small to analyze directly, the size and isotope

composition of the processing blank is instead constrained by analyzing standards of known and distinct  $^{14}C$  content in a similar fashion as the samples (Pearson et al., 1998; Shah and Pearson, 2007; Santos et al., 2007; Mollenhauer and Rethemeyer, 2009; Ziolkowski and Druffel, 2009; Lang et al., 2013). As has been observed with other analyses of small amounts of organic carbon, the standards analyzed by the WCO method had  $F^{14}C$  contents similar to that of the powdered standards at high concentrations. At lower concentrations, these values converge towards the isotope signature of the blank (Figure 1).



**Figure 2.** Corrected  $F^{14}C$  values of phthalic acid standards (upper plot,  $F^{14}C < 0.002$ ) and sucrose standards (lower plot,  $F^{14}C = 1.053 \pm 0.003$ ) vs  $\mu g C$ . Individual analyses from sequence C130304CM1G (empty squares), C130419G (black triangles) and C140708SL1G (gray triangles) are plotted with error bars representing the propagated error of analyses.

The data from the standards were used to calculate the size and isotope composition of the blank for each suite of samples (Table 1). Processing blanks from the three different runs ranged from

$0.68 \pm 0.26$  to  $1.05 \pm 0.23 \mu g C$  with  $F^{14}C$  values of  $0.170 \pm 0.051$  to  $0.274 \pm 0.151$ . The size of the blanks is

similar to the contribution of extraneous carbon from CTC designed for small (<25 µg C) samples. For example, Santos et al., 2007 determined the blank associated with closed tube combustion on their system using <sup>14</sup>C-free coal and modern OX-1 to be 0.2 – 1 µg of modern and 0.1-0.5 µg of <sup>14</sup>C-free carbon.

For the environmental samples analyzed here, with concentrations of 200 – 1700 µM, the blank contributed ~0.6 – 4.6% of the total measured carbon. Analytical approaches that use larger sample volumes have a similar contribution of the blank to the amount of carbon analyzed since they are designed for samples with much lower DOC concentrations. The blanks of an improved UV Oxidation method are reported to be < 2 µM using a 1 L reaction vial (Beaupré et al., 2007). For even the lowest concentrations of seawater dissolved organic carbon of 36 µM this would contribute only 5% of the total measured C.

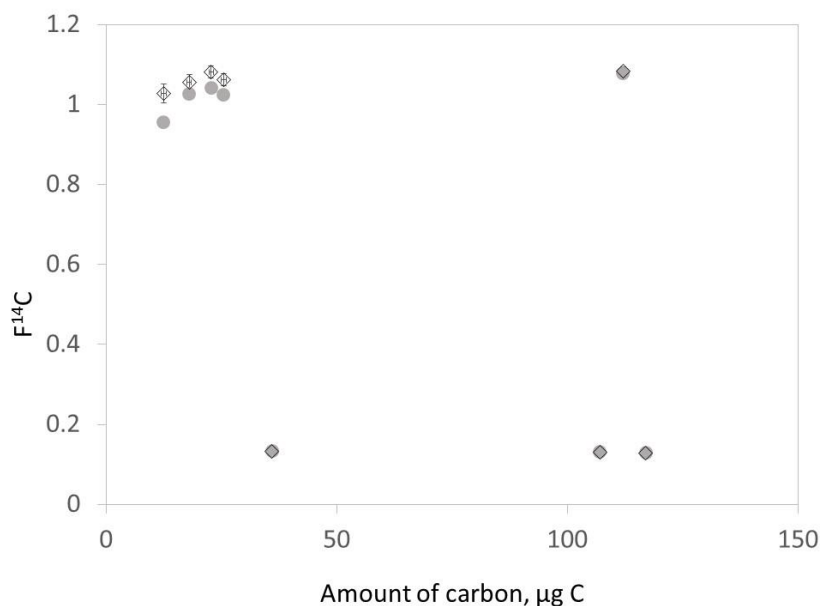
The variability in size and composition of the blank emphasizes the importance of determining the processing blank independently for each suite of analyses. This variability could be caused by the introduction of small amounts of carbon to the water, vials, reagents, user error, or instrument variability. The relatively large number of samples that can be processed simultaneously by the WCO method makes the analysis of 10-12 standards for each run feasible, and is strongly recommended.

**Table 1.** Composition of the WCO processing blank determined for each batch of samples, as determined using two standards (sucrose and phthalic acid)

Sequence Name	Date	#Sucrose standards	#Phthalic standards	Blank µg C	± 1 σ	F <sup>14</sup> C <sub>blank</sub>	± 1 σ
C130304CM1G	03/04/2013	3	3	1.05	0.23	0.170	0.051
C130419G	04/19/2013	5	5	0.70	0.34	0.261	0.171
C140708SL1G	07/08/2014	5	4	0.68	0.26	0.274	0.151

#### *Environmental Samples*

Five freshwater samples were analyzed by the current WCO method and, after correcting for the processing blank associated with each run, had measured F<sup>14</sup>C values from 0.128 ± 0.003 to 1.082 ± 0.015 (Table 2). In both the modern and <sup>14</sup>C-free samples, the absolute errors translate to a similar relative percent error (1.8% vs 1.5%).



**Figure 3.** Comparison of  $F^{14}C_{\text{meas}}$  (gray circles) and  $F^{14}C_{\text{corr}}$  (empty triangles) vs  $\mu\text{g C}$ . Error bars are either instrument error ( $F^{14}C_{\text{meas}}$ ) or propagated error ( $F^{14}C_{\text{corr}}$ ). For samples with large amounts of carbon and/or  $F^{14}C$  values similar to the processing blank (0.170 – 0.274), the marker points overlap. Modern samples with low amounts of carbon required the largest absolute corrections to account for the presence of extraneous carbon during processing.

The reproducibility of the analysis was determined by analyzing two samples multiple times in the same preparation run and in different preparation runs. The older permafrost-fed stream had an average  $F^{14}C$  value of  $0.130 \pm 0.002$  ( $n = 3$ ), with a variability similar to the propagated measurement error associated with each individual analysis. Replicates for the modern Fraser River sample GRO000019 yielded an average  $F^{14}C$  of  $1.069 \pm 0.019$  ( $n = 2$ ), which has a variability somewhat higher than the error associated with the individual analyses. For comparison, the

average  $F^{14}C$  of replicates of the same modern sample analyzed by UV-oxidation was  $1.071 \pm 0.011$  ( $n = 2$ ). The larger differences in reproducibility in the modern samples likely reflects both the lower amounts of carbon analyzed in the Fraser River sample (18.0 – 22.8  $\mu\text{g C}$ ) compared to the permafrost stream sample (31.5 – 117  $\mu\text{g C}$ ), as well as a greater influence of the processing blank ( $F_m \sim 0.2$ ) on the more modern samples (Figure 3; Table 2). Larger absolute corrections must be made to the lower concentration, modern samples.

For GRO000019, the blank correction leads to values

that disagree more, not less, with each other. In this case,  $F^{14}C_{\text{meas}}$  on the two dates is  $1.041 \pm 0.011$  and  $1.027 \pm 0.013$ , a difference of 0.14 that is within approximately one standard deviation while  $F^{14}C_{\text{corr}}$  is  $1.082 \pm 0.015$  and  $1.056 \pm 0.019$ , a difference of 0.026 or greater than one standard deviation. The small number of replicates make it difficult to state with certainty the underlying cause of this observation. One possibility is that the size of the blank has been overestimated, particularly for the samples analyzed in March 2013, when fewer processing standards were used. In the current method the size of the blank has been determined using only pure compounds of known isotopic value. One approach to improving reproducibility between analytical runs would be to analyze an environmental sample of constant and well-known composition, similar to working standards used to correct for drift in stable isotope analyses, or the deep ocean water



provided for dissolved organic carbon concentration analysis. The regular use of an environmental working standard would also allow calibration of  $^{14}\text{C}$  of DOC values across laboratories using multiple different methods.

**Table 2.** Environmental samples analyzed by WCO method

Sequence	Date	ETH #	Sample Label	Analyzed $\mu\text{g C}$ (%recovery) <sup>a</sup>	$\text{F}^{14}\text{C}_{\text{meas}}$	$\pm 1 \sigma$	$\text{F}^{14}\text{C}_{\text{corr}}$	$\pm 1 \sigma$
C130304CM1G	03/04/2013	50006.1.1	GRO000016	25.5 (101%)	1.025	0.011	1.062	0.014
C130304CM1G	03/04/2013	50007.1.1	GRO000018	12.5 (87%)	0.956	0.013	1.028	0.023
C130304CM1G	03/04/2013	50005.1.1	GRO000019	22.8 (92%)	1.041	0.011	1.082	0.015
C140708SL1G	07/08/14	56986.1.1	GRO000019	18.0 (92%)	1.027	0.013	1.056	0.019
C130419G	04/19/2013	50471.1.1	Arctic Stream	112 (115%)	1.077	0.010	1.083	0.011
C130419G	04/19/2013	50468.1.1	Permafrost Stream	36 (128%)	0.134	0.004	0.132	0.006
C140708SL1G	07/08/14	56983.1.1	Permafrost Stream	117 (126%)	0.129	0.003	0.128	0.003
C140708SL1G	07/08/14	56984.1.1	Permafrost Stream	107 (115%)	0.131	0.003	0.130	0.003

<sup>a</sup>Percent recovery is the comparison of measured  $\mu\text{g C}$  to the expected  $\mu\text{g C}$ , based on the volume of sample that was oxidized and the DOC concentration as determined by high temperature combustion (see methods).

The yield of  $\text{CO}_2$  generated by WCO was determined by comparing the expected  $\mu\text{g C}$ , based on the concentration of DOC in the sample and the volume oxidized, and the measured  $\mu\text{g C}$ , based on the amount of gas recovered in the AMS GasTight syringe. Yields ranged from 87 – 101% for the Fraser River samples and 115 – 128% for the Arctic samples (Table 2). Lower recoveries for the Fraser River samples may be due, in part, to incomplete stripping of the  $\text{CO}_2$  from samples with larger water volumes ( $> 4 \text{ mL}$ ). The  $>100\%$  recoveries observed with the Arctic samples by WCO was similar to the values determined by closed tube combustion; the Permafrost Stream sample had a recovery of 113%. These values may therefore point to an issue inherent to these particular samples, e.g. that additional carbon was added after they were analyzed for DOC concentrations but before they were analyzed for  $^{14}\text{C}$  content by WCO and CTC. Alternatively, since the recoveries are based on the amount of gas trapped in the GasTight syringe, the presence of an interfering gas such as  $\text{SO}_2$  could also result in the higher-than-expected values.

The  $\text{F}^{14}\text{C}$  values of these five samples were also assessed by alternate means for comparison to the current method (Table 3). The three modern riverine samples had been previously analyzed using UV-oxidation at NOSAMS. The  $\text{F}^{14}\text{C}$  values determined by the new WCO method had  $\text{F}^{14}\text{C}$  values that were lower than the NOSAMS values by -0.002 to -0.065 (Table 3). Two additional samples, one with a modern and one with an ancient  $^{14}\text{C}$  signature, were analyzed by both WCO and by CTC at ETH. Values determined by the WCO method differed by +0.012 and -0.034, respectively.

305 The offset between the values generated by the WCO method and the other two methods could  
 306 have multiple potential sources that are difficult to constrain at this time. The largest offset of 0.065  
 307 is observed for Fraser River sample GRO000018, which has the lowest DOC concentration (199  
 308  $\mu\text{M}$ ) and the lowest amount of carbon analyzed by WCO (12.5  $\mu\text{g C}$ ). At these low amounts of  
 309 carbon, the precision of the AMS measurement is somewhat decreased and could contribute to the  
 310 offset. Additionally, this sample was analyzed with only six standards (3 phthalic acid, 3 sucrose)  
 311 and therefore the blank was less precisely constrained than for other samples. Finally, minor  
 312 differences in the sample itself may have arisen during storage. The NOSAMS analyses were  
 313 performed in 2010, shortly after sample collection. The WCO of sample aliquots that were  
 314 collected at the same time into different containers were analyzed ~3 years later.  
 315

**Table 3.** Summary comparison of environmental samples analyzed by wet chemical oxidation, UV-oxidation, and quartz tube combustion. For  $\text{F}^{14}\text{C}$ , propagated errors incorporate both the measurement error and the correction for the presence of the processing blank. In cases where a sample was analyzed multiple times, the standard deviation of multiple analyses is reported. N.d. is not determined.

Sample Name	DOC conc. ( $\mu\text{mol C/L}$ ) <sup>a</sup>	Wet Oxidation (ETH)		UV Oxidation (NOSAMS)		Freeze-dried (ETH)		Diff- erence
		$\delta^{13}\text{C}$ (‰)	$\text{F}^{14}\text{C}$	$\delta^{13}\text{C}$ (‰)	$\text{F}^{14}\text{C}$	$\delta^{13}\text{C}$ (‰)	$\text{F}^{14}\text{C}$	$\text{F}^{14}\text{C}$
Arctic Stream	2032	-27.1	$1.083 \pm 0.010$			n.d.	$1.071 \pm 0.011$	0.012
Permafrost Stream	7828	-26.6	$0.130 \pm 0.002$ (n = 3)			n.d.	$0.164 \pm 0.004$	-0.034
GRO000016	395	-27.7	$1.062 \pm 0.014$	-27.3	$1.090 \pm 0.004$			-0.028
GRO000018	199	-29.1	$1.028 \pm 0.023$	n.d.	$1.093 \pm 0.004$			-0.065
GRO000019	546	-26.7	$1.069 \pm 0.019$ (n = 3)	-26.6	$1.071 \pm 0.010$ (n = 2)			-0.002

<sup>a</sup>Data determined by Shimadzu TOC-V analyzer; from Voss et al., 2015 and Mann et al., 2015.

316 Difference in values may also arise between those analyzed by WCO, UV-Oxidation, and freeze-  
 317 drying as a result of variable amounts of purging time. While 5 mL samples are purged for 8  
 318 minutes in the WCO method, the larger volume samples analyzed by UV-oxidation are purged for  
 319 >1 hour. In the quartz-tube combustion method, samples are freeze-dried then subjected to vapor  
 320 phase acidification. While each approach will fully remove inorganic carbon, these different  
 321 methodological approaches may strip different proportions of small semi-volatile organic  
 322 compounds. Several studies have demonstrated that compounds such as formate and acetate are  
 323 partially, but not completely, removed in acidified samples purged with a gas (Barcelona et al.,

1980, Lang et al., 2010). Presumably other small organic molecules with similar attributes will behave similarly.

Finally, differences may arise due to the capability of the different oxidant approaches to convert particularly recalcitrant organic molecules to CO<sub>2</sub>. While concentrations of DOC determined by WCO are identical to those determined by high temperature combustion (Benner and Strom, 1993; Sharp et al., 1995), incomplete oxidation of particularly unreactive molecules cannot be ruled out. Using a UV oxidation system, Beaupré et al. (2007) demonstrated that seawater DOC is converted to CO<sub>2</sub> as a continuum, with later reacting recalcitrant components depleted in <sup>14</sup>C relative to the bulk.

#### *Assessment*

These initial tests demonstrate the utility of a WCO approach for determination of <sup>14</sup>C contents of DOC, although additional improvements could further expand its efficacy and applicability. One advantage of this method is that the preparation time is relatively short, allowing for higher throughput than UV-oxidation or, in some cases, freeze-drying. This is particularly so when the AMS is equipped with an autosampler that can rapidly introduce the sample to a CO<sub>2</sub> gas accepting ion source. In addition to simply being able to process more samples in a single day, the current method also simplifies the concurrent analysis of multiple processing standards over a well-controlled concentration range. On a vacuum line, there are frequently fewer than 10 ports available for the quartz tubes used for CTC, making the preparation of a large number of standards per batch overly time consuming. Additionally, preparing standards for CTC in amounts of <25 µg C can be challenging due to the difficulty in weighing out such small amounts of a powdered standard into the quartz tubes. Instead, larger standards (1 mg) are often combusted and subsequently split into smaller aliquots of gas for analysis (Santos et al., 2007). Because the standards for the WCO method are prepared from a concentrated liquid stock, a precise volume can be easily distributed by pipette.

A second, less obvious, advantage of this approach is that it significantly decreases the potential for cross-contamination of samples, particularly those that have inadvertent contamination from radiocarbon tracers. While great care must still be taken to ensure that samples are not contaminated with tracer <sup>14</sup>C, the WCO method minimizes the damage that can result from a contaminated sample. Each sample is processed and oxidized independently using single-use, disposable glassware. There is a risk of cross-contamination during the sparging step, as the same needle is used to purge each sample. However, replacing a contaminated needle is significantly less costly and time consuming than cleaning numerous components of a vacuum or graphitization line. Once oxidized, the CO<sub>2</sub> is automatically transferred from the vial into the AMS. If the operator of the AMS notices a ‘hot’ sample the run can be immediately terminated, precluding subsequent contamination of later samples. Some carry-over does exist on the AMS system itself, most likely related to the gas lines, water trap, and zeolite traps. Repeat injections of <sup>14</sup>C-free CO<sub>2</sub>

and sparging with helium overnight removes this contamination without the need to disassemble the autosampler or replace the lines. It is because of these attributes that researchers at ETH-Zürich have adopted WCO as the oxidation approach as a screening tool when identifying  $^{14}\text{C}$  contamination (McIntyre et al., 2014).

Future developments should focus on expanding the analysis to saline samples and improving precision; both improvements could potentially be accomplished by increasing sample volume. Interference of  $\text{Cl}^-$  ions with the oxidation currently limits the analysis to freshwater samples, precluding the analysis of seawater. Instruments that use sodium persulfate for oxidation for the  $^{13}\text{C}$  analysis of DOC have overcome this challenge in part by increasing the amount of oxidant relative to sample (Osburn and St-Jean, 2007), which may also provide a solution for this WCO method. The second challenge is the volume limitation imposed by using 12 mL Exetainer® vials. Because the  $\text{CO}_2$  is subsampled from the headspace, the total liquid volume (sample + oxidant) is limited to approximately 7 mL maximum. Increasing the volume of the sample analyzed would allow more  $\text{CO}_2$  to be introduced to the AMS, improving counting statistics and therefore instrument precision. The additional carbon would simultaneously decrease the influence of the blank and further improve the quality of the data. Larger sample vials have been used for dissolved inorganic carbon on this AMS (Molnar et al., 2013) and could potentially be adapted for use with the WCO method.

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